SYNOPSIS

on

“Antimicrobial and Enzymatic activities of Bacteria isolated from the soil of Darbhanga District”.

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L.N. Mithila University, Darbhanga

Submitted by

Md. Muzaffar Ansari

Research Scholar(PAT-2021-22)

University Dept. of Botany

L.N. Mithila University,

Darbhanga -846004

Under the Supervision of

Dr. Khwaja Salahuddin

Assistant Professor

University Dept. of Botany

L.N. Mithila University,

Darbhanga-846004

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Introduction

Bacteria are indispensable organisms for producing enormous antibiotics and enzymes. Bacteria are known for producing secondary metabolites which are produced from microorganisms but these do not act against same microorganism instead they either kill or inhibit the growth of harmful microorganism. Most of the bacteria are found in the soil where these are isolated for ascertaining the antimicrobial and enzymatic properties (Salahuddin *et al*, 2011). The problem of resistance against the antibiotics in bacteria increases day by day so there is urgent need to search new antibiotics. Soil bacteria have played significant role in the discoveries of antibiotics. Enzymes are chemical substances which accelerate the rate of reaction by decreasing the activation energy. There are so many microbes which haveindustrial applications. Actinomycetes constitute a formidable group of industrially important microorganisms that have been explored to produce thermostable enzymes. Starch digestion is mainly done through the enzymatic method. Degradation of starch is carried out mainly and commonly by the action of amylolytic enzyme of bacterial origin (Fossi *et al*, 2005). α-Amylase has been derived from several fungi, yeast, bacteria and actinomycetes. It is used in food, textile, baking, pharmaceutical and detergent industries. It is also used in starch processing industry to convert starch to high fructose. The enzymes are extracted and estimated by standard procedure.

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Review of Literature

Soil microbes such as *Streptomyces, Actinomyces* and *Pseudomonas* have been widely exploited for beneficial use. Cellulose is the most abundant organic compound in the world and consists of glucose units linked by β-1,4 glycosidic bonds in a linear mode (Salahuddin *et al*, 2012). Microbial enzymes are preferred and present a wide spectrum of characteristics that enable them indispensable for quite specific applications as reported by Asgher *et al*, (2007). Ashwini *et al* (2011) documented that Actinomycetes constituted a formidable group of industrially important microorganisms that have been explored for the production of thermostable enzymes. As per the report of Salahuddin *et al*, (2011) the number and species of microbes in soil depend on environmental condition like nutrient availability, soil texture, soil pH, soil moisture. α-amylase enzyme is also used in starch processing industry to convert starch to high fructose.

Aims and objectives

1. Isolation of microbes from the different rhizoplanic and rhizospheric areas of Darbhanga District.
2. Isolation of Pure culture of bacteria with the help of plating techniques
3. Cultural and physiological characterization of microbes
4. Antibiotics sensitivity test based on the zone of inhibition.
5. Detection of enzymes
6. Extraction of enzymes in crude form
7. Estimation of enzymes by assaying technique

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Methodology and Plan of works

1. **Microorganism:**

Soil samples will be randomly collected from different locations in Darbhanga District of Bihar, India from rhizoplane of herbaceous plants. The mesophilic strains of *Streptomyces, Actinomyces, Pseudomonas* will be isolated on different media by using serial dilution technique.

2. **Culture media:**

The following media will be used for isolating mesophilic strains of soil microbes as well as for the extraction of enzyme. The pH of the media will be adjusted to between 6.8 and 7.2 either by addition of 1 N NaOH or 1 N HCl. Then the media will be autoclaved at 121°C for 15 min.

- The Czapek-Dox Agar Medium (CAM) contains 2.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4$. $7H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4.7H_2O$, 30.0 g of Sucrose, 15.0 g of agar and 1000 mL of distilled water.
- Starch Agar Medium (SAM) consists of 2.0 g of soluble starch, 5.0 g of peptone, 3.0 g of beef extract, 15.0 g of agar and 1000 mL of distilled water.

3. **Culture conditions:**

To observe the effect of different culture conditions on α-amylase and cellulase enzyme production, the investigation will be conducted using liquid starch medium at varying incubation periods (24, 48, 72, 96 and120 h), pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and temperatures (45, 50, 55, 60 and 70°C). The effect of each factor on enzyme production and reducing sugars will be observed. The effect of different carbon and nitrogen sources on α-amylase nad cellulase production will be investigated in liquid medium.

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4. **Detection of enzyme:**

   This will be done with Czapek-Dox agar medium containing carboxy methylcellulose (CMC) as carbon source and subjected to autoclaving at 121°C for 15 min. Later, the microorganism will be inoculated on Petri plate. Then the inoculated plate will be subjected to incubation at 45°C in an inverted position for3 to 5 days. The plates will be flooded with 1% aqueous solution of hexadecyltrimethyl ammonium bromide. This reagent will precipitate intact CMC in medium. Now the clear or transparent zones around the colonies of actinomycete will likely be formed which indicated the degradation of CMC by the production of cellulase enzyme.

5. **Antibiotic sensitivity:**

   Thick spore suspensions from the desired strains will be mixed with cooled (45-50^{o}C) molten medium and then poured into sterilized Petri plates. The discs containing the different amounts of antibiotics will be placed at specified marked position of the plates and then subjected to incubation for 48 h at 37^{o}C. The zone of inhibition of these different antibiotics could thus be measured.

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Signature of
Research Scholar
Md. Muzaffar Ansari
M.sc. Botany
PAT-2021-22, Roll- 11
PAT ROLL- 22020041

Signature of
Supervisor
Dr. Khwaja Salahuddin
Assistant Professor
University Dept. of Botany
(L.N.M.U.,Darbhanga)